IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of :

Shigenori OHKAWA et al. :

Serial No. 10/069,180 : Group Art Unit 1624

Filed: February 15, 2002 : Examiner: T. MCKENZIE

For: TRICYCLIC DIHYDROBENZOFURAN DERIVATIVES, PROCESS FOR THE PERPARATION THEREOF AND AGENTS

DECLARATION UNDER RULE 1.132

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Tadatoshi HASHIMOTO, citizen of Japan and residing in Osaka-fu, Japan declare and say that:

1. I graduated from Faculty of Pharmaceutical Sciences, the Kumamoto University, Japan, in March, 1979, and finished the master course in Pharmaceutical Science of the graduate school of the same university in March 1981. I was awarded a Ph. D. degree in pharmaceutics at the same university in 1992.

2. Since April, 1981 up to this time, I have been an employee of Takeda Chemical Industries, Ltd., the assignee of the above-identified application, and engaged in research work in the field of Pharmacology. At present, I am a Research Head of Pharmacology Research Laboratories I, Pharmaceutical Research Division of said company.

3. I am a member of the Japanese Pharmacological Society.

4. I am one of the co-inventors of the above-identified application and am familiar with the subject matter thereof.

5. I have read and understood the Office Action mailed August 7, 2003.

6. In order to show that the claimed compounds in the above-identified application have the desired lipid peroxidation inhibitory activity, the following experiment has been done under my direction.

Experiment

Lipid peroxidation inhibitory activity of the compounds obtained in Examples in the above-identified application was determined according to the same manner as that in Experiment disclosed at pages 188 to 190 of the specification of the above-identified application.

Namely, rat cerebral cortices were obtained after decapitation, homogenized in an ice-cooled phosphate saline buffer (50 mM pH 7.4) (Nichion Microhomogenizer, S-310E), centrifuged at 10,000 g for 10 minutes (Hitachi CF15D type, RT15A6 Anglerotor), and the supernatant was used in a test. This supernatant was diluted 3-fold with the same buffer. To this 1 mL were added 10 µL of test drugs dissolved in dimethyl sulfoxide (DMSO) to the final concentration of 0.0125, 0.025, 0.05, 0.10, 0.20, 0.40, 0.80 and 1.60 µM, respectively, which was incubated at 37°C for 30 minutes. The reaction was stopped

by addition of 200 µL of 35% perchloric acid, and centrifuged at 13,000 g for 10 minutes. To 1 mL of this supernatant was added 0.5 mL of 2-thiobarbituric acid (500 mg/100 mL) dissolved in 50% acetic acid, heated to boil at 95°C for 15 minutes, which was determined by the absorbance at 532 nm. An inhibition rate was obtained from an amount of produced lipoperoxide at each concentration of the compound and the amount of lipoperoxide in a DMSO-added group, and IC_{50} value of a compound was obtained from the inhibition rate.

The results are as follows:

Example number	IC_{50} (µM)
1	0.075
3	0.27
4	0.025
9	0.26
11*	0.067
14	0.028
15	0.081
16	0.046
18*	0.36
19	0.065
20	0.36
21	0.060

*: The data disclosed in Table 2 in page 190 of the specification of the above-identified application.

It is believed that the above experimental results fully support the lipid peroxidation inhibitory activity of the compounds claimed in the above-identified application.

7. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

This 21 day of October, 2003

Tadatoshi Hashimoto

Tadatoshi HASHIMOTO